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Multifaceted determinants of host specificity in an aphid parasitoid

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Abstract The host specificity of insect parasitoids and herbivores is thought to be shaped by a suite of traits that mediate host acceptance and host suitability. We conducted laboratory experiments to identify mechanisms shaping the host specificity of the aphid parasitoid *Binodoxys communis*. Twenty species of aphids were exposed to *B. communis* females in microcosms, and detailed observations and rearing studies of 15 of these species were done to determine whether patterns of host use resulted from variation in factors such as host acceptance or variation in host suitability. Six species of aphids exposed to *B. communis* showed no signs of parasitism. Four of these species were not recognized as hosts and two effectively defended themselves from attack by *B. communis*. Other aphid species into which parasitoids laid eggs had low suitability as hosts. Parasitoid mortality occurred in the egg or early larval stages for some of these hosts but for others it occurred in late larval stages. Two hypotheses explaining low suitability were investigated in separate experiments: the presence of endosymbiotic bacteria conferring resistance to parasitoids, and aphids feeding on toxic plants. An association between resistance and endosymbiont infection was found in one species (*Aphis craccivora*), and evidence for the toxic plant hypothesis was found for the milkweed aphids *Aphis asclepiadis* and *Aphis nerii*. This research highlights the multifaceted nature of factors determining host specificity in parasitoids.

Keywords Host range · Specialization · Preference-performance hypothesis · Oviposition · *Binodoxys communis*

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Introduction

The host range of insect parasitoids and herbivores may include only a single species for extreme specialists or it may include numerous species over a broad taxonomic range for generalists (Futuyma and Moreno 1988; Shaw 1994). The number and taxonomic diversity of species in the host range defines host specificity and this has important implications for speciation and radiation (Futuyma and Moreno 1988), community structure (Memmott et al. 1994) and the ability of insects to invade novel habitats and use novel host species (Andow and Imura 1994; Novotny et al. 2003). For parasitoids and herbivores, both host taxonomy and ecology are known to influence host specificity, as is shown by a number of compilations of field and laboratory host records (e.g., Askew 1994; Stireman et al. 2006). For instance, seminal work on leafminer parasitoids has shown that some parasitoid species specialize on certain taxonomic groups (genera or subfamilies) of leafminers, while other parasitoid species attack leafminers from disjunct taxonomic groups (various orders), as long as all of these leafminers feed upon the same tree species (Askew 1994). The behavioral and physiological mechanisms that underlie such patterns are much less studied, however. Thus, when host specialization

is discerned from field records, it is typically not known whether unsuitable hosts are attacked unsuccessfully, or conversely whether potentially suitable hosts are not attacked (Morehead and Feener 2000). Also, when broad host ranges are found in the field, it is often not clear whether some hosts are preferred or more suitable than others.

In parasitoids, host specificity is mediated by both host recognition and acceptance by the adult female parasitoid (Godfray 1994) and by the suitability of the host for parasitoid development (Pennacchio and Strand 2006). Described as the preference-performance hypothesis in phytophagous insects by Jaenike (1978), host preference in parasitoids tends to correlate with fitness gained from the host (van Alphen and Vet 1986; Driessen et al. 1991; Kraaijeveld et al. 1995; Chau and Mackauer 2001), although very low-quality hosts may also be accepted (Janssen 1989; Heimpel et al. 2003). In addition, host defensive behaviors can limit the ability of parasitoids to utilize even highly preferred hosts (Gross 1993; but see De Farias and Hopper 1999).

Physiological suitability of the host for immature parasitoids is an important determinant of parasitoid host specificity (Godfray 1994; Pennacchio and Strand 2006). Some hosts sequester secondary plant metabolites making them unsuitable for parasitoid development (Ode 2006; Behmer 2009). Immune resistance mediated by host haemocytes can lead to encapsulation of parasitoid eggs as well (Pennacchio and Strand 2006) and in some aphid and weevil species, resistance to parasitoids can be mediated by endosymbiotic bacteria (Oliver et al. 2003, 2005).

Behavioral and physiological determinants of host specificity do not constitute mutually exclusive hypotheses for parasitoid specialization. However, few studies have encompassed these mechanisms for a suite of potential host species for a single parasitoid species (Driessen et al. 1991; Brodeur et al. 1996; Antolin et al. 2006). To evaluate the effects of both behavioral and physiological mechanisms on host specificity for single parasitoid species and test the hypothesis that higher-quality hosts (i.e., those allowing the highest offspring production) are preferred over lower-quality hosts, we conducted laboratory experiments on *Binodoxys communis* Gahan (Hymenoptera: Braconidae), a parasitoid that is native to China but has been released against the invasive aphid pest *Aphis glycines* Matsumura (Hemiptera: Aphididae) in North America in 2007 (Wyckhuys et al. 2007a). Our experiments aimed to: (1) identify high- and low-quality host species for *B. communis*, (2) characterize the parasitoid and host behaviors that could mediate host acceptance, and (3) compare the ability of *B. communis* to complete development in various host species. Two hypotheses explaining low suitability were investigated in separate experiments: the presence of endosymbiotic bacteria conferring resistance against parasitoids, and aphids feeding on toxic host plants. The aphids we tested include species that are closely versus distantly related, on the same versus different host plants, and represent new versus old associations with *B. communis*. These experiments reveal that host specificity in this parasitoid is shaped by a multifaceted array of behavioral and physiological factors, ranging from host defense and parasitoid acceptance to endosymbiont- and host-plant-mediated disruption of host suitability.

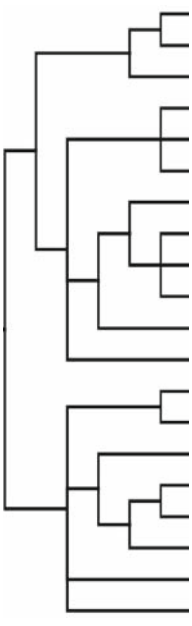
Materials and methods

Insects

Twenty aphid species were tested as hosts, all of which were reared in the Minnesota Department of Agriculture/Minnesota Agricultural Experiment Station (MDA/MAES) Quarantine Facility on their respective host plants at 25°C, 65% relative humidity (RH) and 16:8-h light:dark (L:D). Table 1 gives the aphid species, host plants, and phylogenetic relationships among the aphid species, as well as which aphid species were included and the sample sizes in each of the five experiments reported. These aphid species were chosen because they cover a broad phylogenetic range of hosts and they include four species native to North America as part of a project to determine the potential risk of introduction of *B. communis* to control soybean aphid (Wyckhuys et al. 2007a, b; 2009). More information on the aphids used in this study can be found in Blackman and Eastop (2000, 2006). All records of *B. communis* are from China, Japan and Korea (Chen and Shi 2001; Takada 2002). Thus the four North American aphid species represent new associations for *B. communis*, and the other 16 aphid species represent potential old associations for *B. communis*, based on known aphid distributions (Blackman and Eastop 2000, 2006).

B. communis is a solitary koinobiont endoparasitoid of aphids that is primarily known from cotton aphid, *Aphis gossypii* and soybean aphid, *A. glycines* (Wyckhuys et al. 2007a). Females grasp host aphids with two pairs of abdominal claspers (Desneux et al. 2009) and lay one or rarely 2 eggs per host. Our culture of *B. communis* was initiated with seven males and 33 females from collections of parasitized *A. glycines* in August 2002 near Harbin in the Chinese province of Heilongjiang. Voucher specimens of progeny from the material collected in China are stored at the USDA—Beneficial Insect Introductions and Research Laboratory (BIIRL) in Newark, Delaware, USA. This culture was maintained for 26 generations at BIIRL and then shipped to the MDA/MAES Quarantine Facility where the colony was maintained for approximately 80 generations on *A. glycines* on soybean (25°C, 65% RH and 16:8-h L:D)

Table 1 Aphid species, phylogenetic relationships, host plants (from which aphids were collected and on which they were cultured), and replication in experiments with *Binodoxys communis*

Phylogeny	Species	Host plant species	Experiments (replication)	Tribe
	*Rhopalosiphum padi* Linnaeus	*Hordeum vulgare*	1(9), 2(10)	Aphidini
	Rhopalosiphum maidis Fitch	*Hordeum vulgare*	1(12), 2(10)	
	Schizaphis graminum Rondani	*Hordeum vulgare*	1(10), 2(49), 3(65), 4(5)	
	Aphis rumicis L.	*Rumex altissimus*	1(11), 2(61), 3(72), 4(5)	
	Aphis craccivora Kock	*Vicia fabae*	1(10), 2(26), 3(81), 4(5)	
	Aphis asclepiadis F. *	*Asclepias syriaca*	1(11), 2(56), 3(69), 4(5), 5(140)	
	Aphis glycines Matsumara	*Glycine max*	1(13), 2(44), 3(69), 4(10)	
	Aphis gossypii Glover	*Gossypium hirsutum*	1(13), 2(57), 3(55), 4(5)	
	Aphis monardae Oestlund *	*Monarda fistulosa*	1(12), 2(51), 3(73), 4(5)	
	Aphis oestlundi Gillette *	*Oenothera biennis*	1(10), 2(58), 3(76), 4(5)	
	Aphis nasturtii Kaltenbach	*Solanum tuberosum*	1(10)	
	Aphis nerii Boyer de Fonscolombe	*Asclepias incarnata*	1(14), 2(49), 3(69), 4(5), 5(157)	
	Myzus persicae Sulzer	*Brassica oleracea*	1(11), 2(11)	Macrosiphini
	Diuraphis noxia Kurdjumov	*Hordeum vulgare*	1(10)	
	Uroleucon leonardi Olive *	*Echinacea purpurea*	1(10), 2(10)	
	Macrosiphum euphorbiae Thomas	*Solanum tuberosum*	1(10)	
	Sitobion avenae F.	*Hordeum vulgare*	1(11)	
	Acyrthosiphon pisum Harris	*Vicia fabae*	1(11), 2(12)	
	Lipaphis erysimi Kaltenbach	*Brassica oleracea*	1(10), 2(10)	
	Aulocorthum solani Kaltenbach	*Solanum tuberosum*	1(11)	

Aphid species—All aphid species are in the family Aphididae and subfamily Aphidinae; Phylogenetic relationships—Phylogeny is from information in von Dohlen et al. (2006), Coeur d'Acier et al. (2007), and K. R. Hopper (unpublished data); From which aphids were collected—All aphids were collected in the field in the vicinity of St. Paul, Minnesota, USA in 2003 with the exception of the Russian wheat aphid, *Diuraphis noxia*, which had been previously reared at the USDA ARS Beneficial Insects Introduction and Research Laboratory in Newark, Delaware, USA, since 1998 and was originally from field collections in Wyoming

Asterisk indicate aphid species native to North America

before the experiments. Parasitized aphids were removed from soybean leaves at the pupal (mummy) stage and kept individually in plastic Petri dishes until the emergence of adult parasitoids. Females were mated within 24 h of emergence and supplied with a droplet of honey diluted in water (80% honey) prior to use in experiments. Parasitoids used for all experiments were 24–48 h old, used only once, and had never been in contact with plants or aphids.

Experiment 1: parasitoid offspring production on single aphid colonies

Female *B. communis* were allowed to oviposit for 24 h on aphids placed on their respective host plants, which were individually potted and covered by plastic cylindrical cages ("microcosms"; diameter 11 cm, height 21 cm; 12 holes with mesh for ventilation). Fifty aphids of mixed instars were placed per plant and allowed to settle for 1 h before the introduction of *B. communis*, and cages were kept in a growth chamber at 16:8-h L:D, 25°C, and 65% RH (see Table 1 for details of sample size). Aphids were monitored daily and newly developed mummies were isolated individually in clear gelatin capsules. We counted the mummies produced per microcosm, and the male and female adults that emerged. The numbers of parasitoid mummies produced per microcosm and the numbers of adults produced (i.e., offspring production) were compared among aphid species using ANOVA with a general linear model (proc glm in SAS/STAT version 9.1; SAS Institute, Cary, N.C.) and means were compared with *t*-tests using probabilities from re-sampling the data (values were permuted randomly among experimental units 20,000 times and the probability of the observed values determined from the distribution generated by these permutations), controlling for experiment-wise error rate at $P = 0.05$ (proc multtest). The data were square-root transformed to homogenize variances. The proportion of adults emerging from mummies was compared among aphid species using ANOVA with a general linear model (proc glm). Proportions were arcsin-square-root transformed. We used log-likelihood goodness-of-fit tests to evaluate the hypothesis that observed sex ratios for *B. communis* on each aphid species differed from 0.5 (Heimpel and Lundgren 2000).

Experiment 2: host acceptability and oviposition

We directly observed parasitoid behavior to determine the capacity of *B. communis* to detect, accept and attack 15 of

the aphid species studied in experiment 1. These species were chosen to represent a mixture of those that did versus did not produce mummies so that behaviors would be observed for both classes of hosts. We also observed aphid defensive behaviors and the effectiveness of these behaviors in preventing parasitism. Finally, we assessed the relationship between aphid defensive behaviors and oviposition success and handling time of *B. communis*.

The substrate for observations was a leaf placed upside down under a binocular microscope. The leaf stem was inserted into a water-filled 1.5-ml microcentrifuge tube. We placed one individual of a given aphid species onto the leaf using a fine brush and allowed it to establish for 5 min. Given that these aphid species vary in size and size may affect acceptance rates (Henry et al. 2006) and effectiveness of behavioral defenses (Gerling et al. 1990), we used aphids of all species equivalent in size to 3rd instar *A. glycines*, the stage preferred by *B. communis* (Wyckhuys et al. 2008). Individual mated *B. communis* females were introduced into a clear plastic dome (diameter 1 cm, height 0.65 cm) and the dome was placed over an individual aphid on a leaf. Observations began when the parasitoid first encountered the leaf. Parasitoids were observed for 5 min, or until a successful ovipositor insertion occurred. On each day of observation, parasitoids were observed on each aphid species with the order of aphid species randomized (see Table 1 for details of sample size).

We recorded parasitoid behaviors as: "antennal contact" (contact with the aphid by at least one parasitoid antenna), "antennal palpation" (antennal palpation of the aphid by the parasitoid), "successful sting" (an ovipositor insertion greater than 3 s and not ending due to aphid defensive behaviors) and "interrupted sting" (all other sting attempts, i.e., abdomen bent underneath thorax with or without an ovipositor contact). We also recorded aphid defensive behaviors as "kick", "rotation", "antennal push" or "cornicle secretion".

These behaviors were used to categorize interactions between each parasitoid individual and each aphid individual. Antennal contact followed by antennal palpation was categorized as "detection"; antennal palpation followed by stinging (whether successful or not) was categorized as "acceptance for stinging", and an aphid sting lasting more than 3 s was categorized as a "successful sting" (as described above). Differences in frequencies of detection, acceptance and successful stinging among aphid species were analyzed with a log-linear model (proc genmod), each individual aphid being scored as detected, accepted, or successfully stung. Then, the proportions of aphids in each category for the parasitoid on *A. glycines* (the collection and rearing host) were compared to the proportions on other aphid species using permuted Fisher's exact tests (proc multtest). Handling time, defined as time between 1st encounter and successful stinging, was compared among aphids with ANOVA on log-transformed data, followed by Tukey's post-hoc test. Stinging time was compared among aphids with a Kruskal–Wallis test. Nonparametric tests were used when assumptions of parametric tests were not met and could not be achieved using transformations. Pairwise correlations among detection, acceptance and successful stinging proportions, and between the successful stinging proportion and mean handling times were tested using weighted linear regression.

The frequencies of aphid defensive behaviors were compared among aphid species using logistic regression (proc genmod). We tested for correlations between the number of each class of defensive behaviors and the number of failures in host detection, acceptance, and interrupted stings over the course of the observation period using linear regression. We also tested for correlations between the numbers of each class of defensive behaviors on the total handling time.

Finally, we used linear regression analyses to investigate separately the effects of host detection, acceptance and the successful sting rate on host suitability, which was itself measured as the rate of complete immature development (experiment 3, below). This could only be done for those nine aphid species which were successfully stung and therefore provided information on host suitability.

Experiment 3: host suitability

We measured survival of parasitoid eggs, larvae and pupae within different host aphid species. Following the behavioral observations described above, aphids having been successfully stung were removed from the observation arena and returned to the host plant enclosed within a plastic clip cage (diameter 3.5 cm, height 1 cm). These replicates were augmented using additional aphids that were observed to be successfully stung by additional *B. communis* following the same procedure as in experiment 2, but without recording parasitoid behavior (see Table 1 for details of sample size). To account for mortality caused by handling, control aphids were handled but not parasitized, and their survival was recorded 7 days later (minimum of 20 individual aphids per species, comparison between species based on a log-linear model). Parasitized aphids were kept on their respective host plants in environmental cabinets at 23 ± 1°C, 60% RH, 16:8-h L:D. Under these conditions, *B. communis* eggs hatch 2–3 days after oviposition. To measure survival of *B. communis* immatures, sub-samples of hosts were dissected under a binocular microscope at 40× magnification either immediately after being stung, or 4 days after being stung. A third sub-sample of stung hosts was retained until 10 days after successful stinging when mummification of the host occurs. Depending on species,

17–29 and 17–31 aphids were dissected for eggs and larvae, respectively, and between 21 and 27 aphids were observed to record mummy formation and subsequent adult emergence.

The proportion of stung aphids that contained eggs and the survival of immature parasitoids from egg to 4th day (larvae), from 4th to 10th day (mummies) and parasitoid adults among the aphid species were fitted to a log-linear model. In addition, proportions of parasitoids found at each developmental stage were compared per aphid species using pairwise Fisher's exact tests (Dunn–Sidak adjustment for multiple comparisons). Deviance from an unbiased sex ratio was assessed as in experiment 1.

Experiment 4: presence of secondary endosymbionts in host aphids

We screened the aphid species tested in experiment 3 (see Table 1) for the presence of two facultative secondary symbionts, *Serratia symbiotica* and *Hamiltonella defensa*, both of which are known to confer resistance in aphids to parasitoids (Oliver et al. 2003, 2005). DNA extractions were done using the Puregene DNA isolation tissue kit (Gentra Systems, Minneapolis, Minn.), and samples were subjected to two diagnostic PCR amplifications per symbiont tested. We used two diagnostic forward primers for each symbiont (*H. defensa*, T1279F, T99F; *S. symbiotica*, R1279F, R250F) and a universal reverse primer (*H. defensa*, 480R; *S. symbiotica*, 1502R), which amplify a fragment of the 16–23S rRNA operon (Russell et al. 2003). PCR amplifications were carried out as described by Russell et al. (2003) except that we used Gotaq Green mix (Promega, Madison, Wis.). PCR products were run on 2% agarose gels, stained in ethidium bromide and visualized with UV light. Bands were cut from the gel, purified using Ultrafree-DA columns (Micron Bioseparations, Billerica, Mass.) and sequenced directly at the University of Minnesota BioMedical Genomics Center. All PCR reactions included a negative control, and amplifications were also run using conserved primers of the cytochrome oxidase I (COI) gene using primers from Simon et al. (1994) to detect false negatives. Samples were scored as negative for symbiont DNA if they did not amplify, if they yielded improperly sized products, or if the PCR product was less than 98% similar to previously described symbionts (based on GenBank blast searches) despite the COI primers resulting in appropriate amplification.

Experiment 5: impact of host plant on host suitability

We compared *B. communis* development on *Aphis asclepiadis* feeding on common milkweed, *Asclepias syriaca*, which produces toxic cardenolides (Martel and Malcolm 2004), and ivyleaf morning glory, *Ipomoea hederacea*. *Ipomoea* spp. plants are known to have constitutive defenses localized in the latex of laticifers (Schadel and Walter 1981) but aphids are able to avoid latex ducts and ingestion of other defense products of these plants (Hull-Sanders and Eubanks 2005). *Aphis asclepiadis* was selected as the host for this experiment because of a high *B. communis* larval mortality rate in this host when feeding on *A. syriaca* (see "Results"). We followed the same protocol described for experiment 3 on the two host plants (replicates for *A. syriaca* and *I. hederacea*, respectively; egg, 21, 25; larvae, 22, 23; mummy, 20, 29). A subset of *A. asclepiadis* were reared for four generations on *I. hederacea* before starting the experiment. Parasitoid survival in the aphids on the two different host plants was analyzed using a log-linear model (proc genmod). Proportions of parasitoids found at each developmental stage were compared per plant species by pairwise Fisher's exact tests (with the Dunn–Sidak adjustment method).

Following the same protocol, we also compared parasitoid survival in *Aphis nerii* that fed either on *Asclepias incarnata* (the plant used in experiment 3) or *A. syriaca* (replicates: egg, 30, 20; larvae, 32, 21; mummy, 34, 20, respectively). *A. incarnata* and *A. syriaca* are low- and medium-constitutive cardenolide plants respectively (Malcolm and Zalucki 1996; Mooney et al. 2008) and *B. communis* was therefore expected to perform better in hosts that fed on *A. incarnata*. Statistical analyses were carried out as described above.

Results

Experiment 1: parasitoid offspring production on single aphid colonies

Numbers of mummified aphids and adult parasitoids differed greatly among aphid species (Fig. 1; mummies, $F_{19,199} = 20.4$, $P < 0.001$; adults, $F_{19,199} = 18.6$, $P < 0.001$). *B. communis* produced the most mummies and adults on *A. glycines*, *Aphis monardae* and *A. gossypii*, and the mean number of mummies did not differ among these species. Fewer mummies were produced on other species in the genus *Aphis*, notably *A. nerii* and *Aphis craccivora*.

Parasitoid emergence rates varied among aphid species ($F_{13,81} = 4.7$, $P < 0.001$). *Schizaphis graminum* gave the highest adult emergence rate (0.96), and the emergence rate from *A. glycines* (0.74) ranked fourth, although these values did not differ significantly. The parasitoid sex ratio was significantly female-biased in *A. glycines*, but significantly male-biased in *A. monardae*, *A. gossypii, A. asclepiadis, Aphis nasturtii* and *Aphis oestlundi* (Fig. 1).

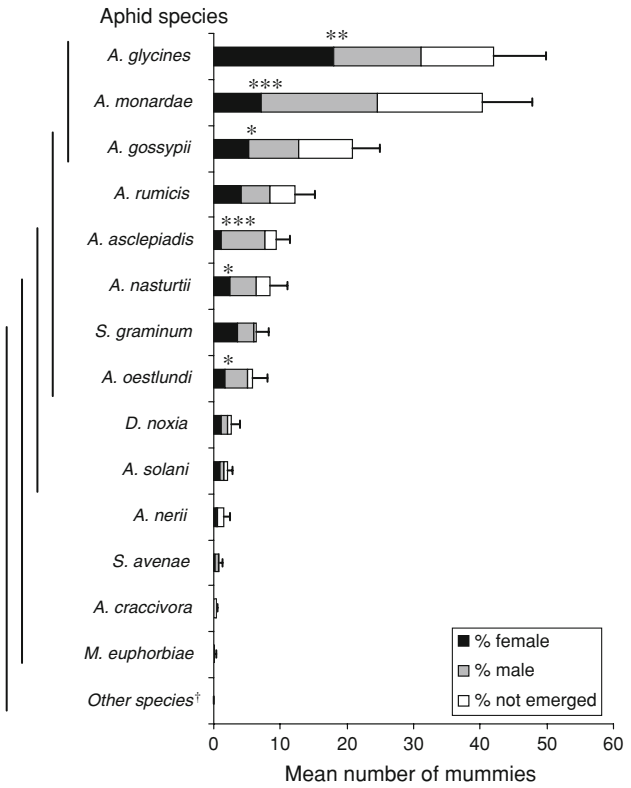


Fig. 1 Mean numbers of mummies and adult female and male offspring produced per plant (experiment 1). Means for species subtended by *lines* do not differ ($P > 0.05$, permuted *t*-tests controlling for multiple comparisons). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ (deviation from a 0.5 sex ratio). *Other species* included *Acyrthosiphon pisum*, *Lipaphis erysimi*, *Myzus persicae*, *Rhopalosiphum maidis*, *Rhopalosiphum padi*, *Uroleucon leonardi*

Experiment 2: host acceptability and oviposition

Aphid species varied in detection by *B. communis* (Table 2; $\chi^2_{14} = 28.97$, $P < 0.001$). Detection proportions ranged from 0.91 to 1.00 for the eight species of *Aphis* tested and for *S. graminum*, which is a fairly suitable host. However, *B. communis* also readily detected *Uroleucon leonardi*, which appears to be a non-host. The remaining five species had low detection proportions, all of which were significantly lower than the value for *A. glycines*.

Aphid species also varied in acceptance for stinging by *B. communis* (Table 2; $\chi^2_{14} = 59.69$, $P < 0.001$). *B. communis* accepted *A. glycines* and *A. monardae* at the highest rate and four other *Aphis* species at rates that did not differ from these species. The parasitoid either did not accept non-*Aphis* species or accepted them at lower rates. Acceptance reflected detection with several important exceptions. First, both *A. gossypii* and *S. graminum* were accepted at lower rates than *A. glycines*. Second, both *Acyrthosiphon pisum* and *U. leonardi*, were detected at high rates but accepted at low rates, and *Rhopalosiphum maidis* was detected at a low rate, but all detected individuals were accepted (weighted linear regression of detection rate on proportion detected that were accepted, all data, $P = 0.018$, $df = 1,13$, $r^2 = 0.36$; excluding *A. pisum*, *U. leonardi* and *R. maidis*, $P < 0.001$, $df = 1,10$, $r^2 = 0.91$). These cases of high detection and low acceptance appear to result primarily from defensive behavior on the part of the aphids (see below).

The proportion of aphids successfully stung varied among aphid species (Table 2; $\chi^2_{14} = 109.80$, $P < 0.001$). The proportion ranged from 0.80 to 0.98 among six species of *Aphis*, with highest proportions on *A. glycines*, *A. monardae*, and *A. oestlundi*. For an additional three species (*Aphis rumicis*, *A. craccivora*, and *S. graminum*), the proportion successfully stung was significantly lower than on *A. glycines* but greater than 50%. None of the remaining six aphid species were stung. Among aphid species that were accepted for stinging, acceptance was highly correlated with successful stinging (weighted linear regression of acceptance rate and proportion of those aphids accepted that were successfully stung; $r^2 = 0.89$, $df = 1,13$, $P < 0.001$).

The shortest handling times were found for *A. glycines* and *A. monardae*, with intermediate handling times for *A. asclepiadis*, *A. nerii* and *S. graminum*, and the longest handling time for *A. craccivora* (Table 2; $F_{8,370} = 4.442$, $P < 0.001$). There was a negative correlation between the proportion of aphids successfully stung and the handling time (weighted linear regression of stinging rate and average handling time; $r^2 = 0.68$, $df = 1,7$, $P < 0.01$), indicating that host species that were the most successfully attacked also took the least time to handle. The stinging times of *B. communis* did not differ among the aphid species that were stung (Table 2; $K = 5.796$, $df = 8$, $P = 0.670$).

The frequency of defensive behaviors differed among aphid species (Fig. 2; likelihood ratio = 94.2, $df = 14$, $P < 0.001$) with *A. nerii* and *U. leonardi* showing significantly higher frequencies of defenses than the other species. Linear regressions between the failure in detection of aphids and defensive behavior events showed that defensive behaviors reduced successful detection (i.e., when antennal contact is followed by antennal palpation) of *A. pisum* ($r^2 = 0.68$, $df = 1,10$, $P = 0.001$). Kicking ($r^2 = 0.48$, $df = 1,10$, $P = 0.013$) and antennal pushing ($r^2 = 0.82$, $df = 1,10$, $P < 0.001$) both reduced detection of *A. pisum*. Defensive behaviors reduced acceptance of *U. leonardi* ($r^2 = 0.81$, $df = 1,8$, $P < 0.001$) and to a lesser extent *A. glycines* ($r^2 = 0.40$, $df = 1,42$, $P < 0.001$) and *A. nerii* ($r^2 = 0.44$, $df = 1,47$, $P < 0.001$). For these three species, kicking also reduced acceptance (*U. leonardi*, $r^2 = 0.54$, $df = 1,8$, $P = 0.016$; *A. glycines*, $r^2 = 0.46$, $df = 1,42$, $P < 0.001$; *A. nerii*, $r^2 = 0.42$, $df = 1,47$, $P < 0.001$). Defense behavior reduced successful stinging

Table 2 Proportions of aphids detected, accepted and successfully stung by *B. communis* when encountering different aphid host species, and mean handling and stinging times per host attacked (in seconds) (experiment 2)

Aphid species[a]	Proportion of aphids detected	Proportion of aphids accepted	Proportion of aphids stung	Mean handling time (s)[b]	Mean stinging time (s)[c]
Aphis glycines	*1.00*	*0.98*	*0.98*	32.45 ± 3.97 a	13.39 ± 1.33
Aphis monardae	0.98	0.98	0.98	32.40 ± 4.54 a	12.07 ± 1.71
Aphis gossypii	0.91	0.81*	0.80*	48.42 ± 6.82 ab	12.05 ± 2.05
Aphis rumicis	1.00	0.92	0.72**	48.28 ± 6.16 ab	10.68 ± 0.72
Aphis asclepiadis	0.96	0.91	0.86	53.97 ± 7.63 b	13.53 ± 2.86
Schizaphis graminum	0.92	0.76**	0.71**	56.02 ± 8.79 b	11.88 ± 1.86
Aphis oestlundi	0.98	0.98	0.97	39.04 ± 4.86 a	14.98 ± 2.31
Aphis nerii	1.00	0.96	0.90	53.06 ± 7.19 b	12.00 ± 1.68
Aphis craccivora	0.92	0.69**	0.54***	111.09 ± 22.04 c	12.80 ± 1.88
Acyrthosiphon pisum	0.67**	0.08***	0.00***	–	–
Lipaphis erysimi	0.30***	0.10***	0.00***	–	–
Myzus persicae	0.27***	0.09***	0.00***	–	–
Rhopalosiphum maidis	0.20***	0.20***	0.00***	–	–
Rhopalosiphum padi	0.30***	0.10***	0.00***	–	–
Uroleucon leonardi	0.90	0.00***	0.00***	–	–

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (significantly different from values of *A. glycines* (*in italics*) (permuted Fisher exact test)

[a] Aphid species are ordered as in Fig. 1 to allow comparison of the results (except for Other species that are ordered alphabetically)

[b] Values followed by the *same letters* are not significantly different ($P > 0.05$, ANOVA followed by Tukey's post-hoc test)

[c] No significant difference between stinging times (Kruskal–Wallis test)

of *A. craccivora* ($r^2 = 0.63$, df = 1,16, $P < 0.001$) with antennal pushing in particular correlated with increased interrupted stings ($r^2 = 0.53$, df = 1,16, $P = 0.001$). Finally, handling time increased with defensive behavior in three species, *A. craccivora* ($r^2 = 0.57$, df = 1,16, $P = 0.002$), *A. nerii* ($r^2 = 0.30$, df = 1,42, $P < 0.001$) and *S. graminum* ($r^2 = 0.34$, df = 1,33, $P < 0.001$), which resulted from kicking behavior (*A. craccivora*, $r^2 = 0.55$, df = 1,16, $P = 0.002$; *A. nerii*, $r^2 = 0.35$, df = 1,42, $P < 0.001$; *S. graminum*, $r^2 = 0.32$, df = 1,33, $P < 0.001$).

Across aphid species, acceptance for stinging was negatively correlated with kicking (Wald $\chi^2 = 17.4, P < 0.001$), but positively correlated with cornicle secretion (Wald $\chi^2 = 7.4$, $P < 0.01$). Successful stinging was negatively correlated with kicking (Wald $\chi^2 = 17.4$, $P < 0.001$) and antennal pushing (Wald $\chi^2 = 6.2, P = 0.01$), but positively correlated with cornicle secretion (Wald $\chi^2 = 11.6$, $P < 0.001$) and rotating (Wald $\chi^2 = 5.3, P = 0.02$). Thus, it appears that stinging attempts elicit cornicle secretions more strongly than they elicit kicking and antennal pushing.

Experiment 3: host suitability

The proportion of successfully stung aphids with eggs varied from ~70% to 90% but did not differ among aphid species (Fig. 3; $\chi^2_8 = 5.75, P = 0.675$). However, the proportions of larvae ($\chi^2_8 = 18.67$, $P = 0.016$), mummies ($\chi^2_8 = 43.22$, $P < 0.001$) and adults ($\chi^2_8 = 50.24$, $P < 0.001$) did differ among aphid species (Fig. 3). The highest survival to adulthood was in *A. glycines* and *A. gossypii* (Fisher's exact tests, all $P > 0.05$). The other aphid species can be grouped based upon the stage at which parasitoid mortality occurred. First, in *A. craccivora,* mortality was high between egg and larval stages ($P = 0.002$). In a second group, aphids feeding on *Asclepias* spp., mortality occurred between the larval and pupal stages (*A. asclepiadis*, $P = 0.004$; *A. nerii,* $P = 0.001$). Finally, in a third group, mortality occurred during/after the pupal stage (*A. oestlundi*, $P = 0.004$; *A. rumicis,* $P = 0.007$; *S. graminum*, $P = 0.001$; *A. monardae,* $P = 0.003$). Aphid mortality from our handling did not differ among species ($\chi^2_8 = 2.64$, $P = 0.954$), nor did aphid mortality from parasitoid stinging differ among species ($\chi^2_8 = 7.97$, $P = 0.436$).

Unlike the sex ratios in experiment 1, which ranged from female to highly male biased, the sex ratio of adult *B. communis* was uniformly female biased on all aphid species in this experiment [from 0.14 (*S. graminum*) to 0.46 (*A. glycines*)]. The sex ratio was significantly different from 0.5 only in the case of *A. gossypii* (0.17) ($G_1 = 5.59$, $P < 0.05$).

None of the regressions testing for effects of detection, acceptance, or stinging on egg-to-adult survivorship

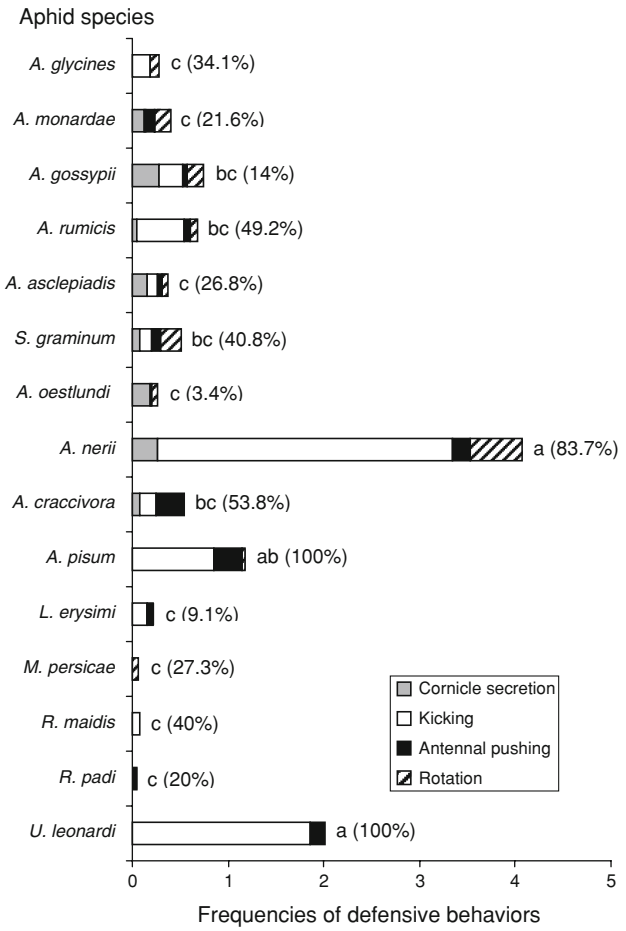


Fig. 2 Frequencies of aphid defensive behaviors over the allotted time period (i.e., 5 min or until successful oviposition), and exhibited by the aphids upon encounter and attack by *B. communis* (experiment 2). *Bars* followed by the *same letter* are not significantly different. Percentages of aphids showing defensive behaviors are indicated *in parentheses*. Aphid species are ordered as in the Fig. 1 (except for Other species, ordered alphabetically) to allow comparison between figures

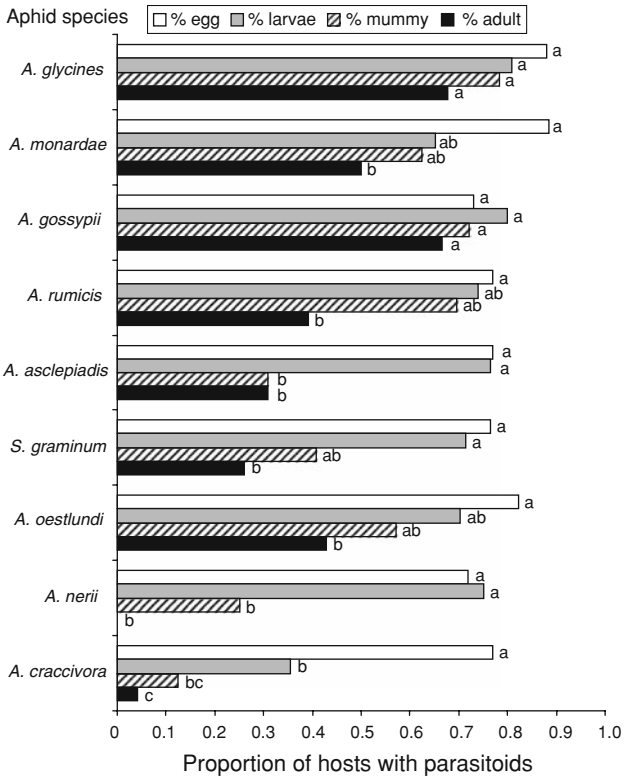


Fig. 3 Proportion of successfully stung aphids that: (1) contained an egg immediately after being stung, (2) contained a larva after 4 days, (3) mummified after 10 days, and (4) produced an adult parasitoid (experiment 3). For each aphid species, *bars* followed by the *same letter* are not significantly different (pairwise Fisher's exact tests with Dunn–Sidak adjustment method). Aphid species are ordered as in Fig. 1

(suitability) for the subset of nine aphid species that were successfully attacked showed significant correlations ($P > 0.15$ for all analyses). This lack of significance was due to one outlier, *A. nerii*, which was stung at a high rate but was unsuitable for the development of *B. communis* (Table 2; Fig. 3). Excluding *A. nerii* led to a significant correlation between successful stinging and suitability (Fig. 4; $P = 0.042$).

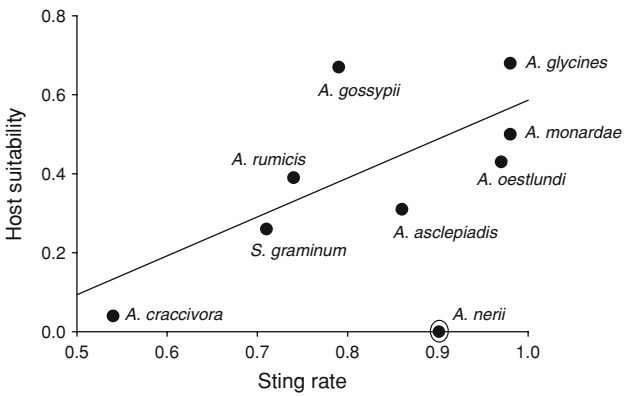


Fig. 4 Relationship between the successful sting rate and the egg-adult suitability of nine aphid hosts of *Binodoxys communis* (experiments 2 and 3). The *regression line* is fit for all aphids except the outlier *Aphis nerii* [suitability = 0.99 (sting rate)—0.40; $r^2 = 0.53$; $P = 0.042$]

Experiment 4: presence of secondary symbionts in host aphids

H. defensa was found in all individuals of *A. craccivora* tested (5/5). Both diagnostic PCR amplifications gave consistent results and identification of the symbiont was confirmed by BLAST searches showing 100% homology with *H. defensa* (GenBank accession no. AY296733). Other aphid species were negative for *H. defensa*, and *S. symbiotica* was not found in any individuals of the nine aphid species screened.

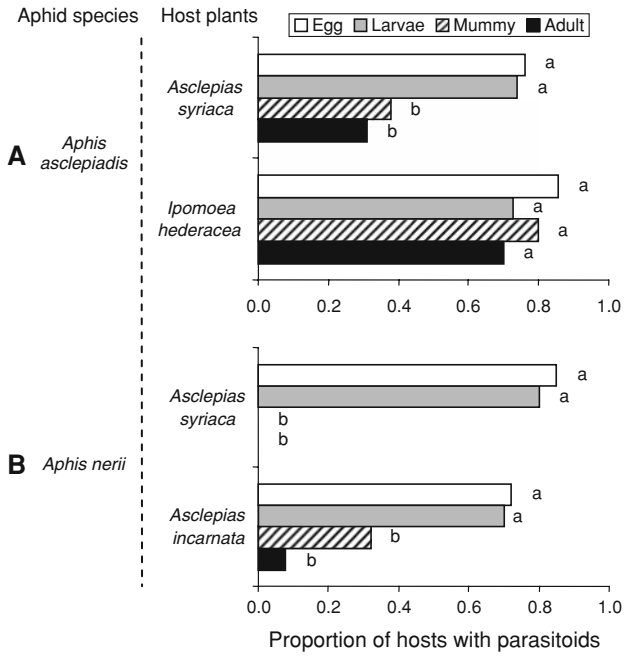


Fig. 5 Proportion of successfully stung aphids [*Aphis asclepiadis* on *Asclepias syriaca* or *Ipomoea hederacea* (*A*), *Aphis nerii* on *Asclepias syriaca* or *Asclepias incarnata* (*B*)] that contained an egg immediately after being stung, a larvae (after 4 days), was mummified (after 10 days), or produced an adult parasitoid (experiment 5). On each host plant, *bars* followed by the *same letter* are not significantly different (pairwise Fisher's exact tests with Dunn–Sidak adjustment method)

Experiment 5: impact of host plant on host suitability

Survival of *B. communis* in *A. asclepiadis* differed significantly between *A. syriaca* and *I. hederacea* (Fig. 5, A; $\chi^2_1 = 4.95$, $P = 0.026$). On *A. syriaca*, the proportion of aphids with parasitoid larvae (74%) was significantly higher than the proportion of mummified aphids (38%) (Fisher exact test: $P = 0.009$). In contrast, high proportions of parasitoids were found in both developmental stages when *A. asclepiadis* fed on *I. hederacea*. Parasitoid survival in *A. nerii* improved when the host was switched from a medium-cardenolide plant (*A. syriaca*) to a low-cardenolide plant (*A. incarnata*) (Fig. 5, B; $\chi^2_1 = 4.42$, $P = 0.035$). Whereas some parasitoids mummified aphids on *A. incarnata*, no parasitoids mummified aphids on *A. syriaca*.

Discussion

We identified a number of factors that mediate host specificity of the aphid parasitoid *B. communis* under laboratory conditions. Successful oviposition was mediated by detection and acceptance of hosts by parasitoids and by aphid defense. Some hosts into which parasitoids oviposited were nevertheless poor hosts for parasitoid development. Host-plant-mediated factors as well as possible resistance conferred by a bacterial endosymbiont contributed to this lack of suitability for three of the hosts, but other factors likely mediate the suitability of aphids as hosts for *B. communis* as well. The willingness and ability of *B. communis* to successfully sting aphid hosts was linked to progeny survival with the exception of one outlier, *A. nerii*, which was stung at a high rate but from which no offspring emerged. The low suitability of this aphid may have been due to its ability to sequester toxic host plant allelochemicals (Rothschild et al. 1970; Malcolm 1989). Given this caveat for *A. nerii*, our results are consistent with the preference-performance hypothesis (Jaenike 1978) and results from some other parasitoid species (van Alphen and Vet 1986; Driessen et al. 1991; Kraaijeveld et al. 1995; Chau and Mackauer 2001).

Host acceptability and oviposition

B. communis failed to detect two aphids from the genus *Rhopalosiphum* and four aphids from the tribe Macrosiphini. The lack of response to *Rhopalosiphum padi* and *R. maidis* cannot be explained by their host plant (Gardner and Dixon 1985) because *S. graminum* was well attacked on the same plant (*H. vulgare*). Some of the suitable hosts put up active, but ultimately ineffective defensive behaviors, as has been found in some other parasitoid–host systems (De Farias and Hopper 1999). Although *A. nerii* had the highest frequency of defensive behaviors, it was successfully stung 90% of the time. In contrast, *A. pisum* and *U. leonardi* appear to escape detection by the parasitoid by performing defensive behaviors, preventing antennal palpation after an initial contact. Thus, while aphid defensive behavior can mediate host specificity of parasitoids, parasitoids are able to circumvent these defenses in some cases. The females of some aphid parasitoids mimic the behavior of aphid-mutualistic ants by tapping aphids frequently with the antennae, thus reducing the risk of defensive behavior (Völkl and Kroupa 1997). The effectiveness of defensive behavior also varies with the aphid instar (Gerling et al. 1990; Wyckhuys et al. 2008) and thus some of the "high-quality" hosts in our study may be inaccessible as larger instars, but we did not address this in our study. Aphid parasitoids may have behaviors adapted to efficiently approach some aphid species but not others, leading to behavioral specialization to these species.

An increase in handling time associated with aphid defensive behaviors was observed for three aphid species. Two of these, *A. nerii* and *A. craccivora*, are poor hosts physiologically, so time spent handling and oviposition within these hosts wastes time as well as eggs (Heimpel et al. 2003). The other, *S. graminum*, is more physiologically suitable, but increased handling time may still decrease fitness under conditions of time limitation.

Suitability for parasitoid development

One potential source of resistance to immature parasitoids in aphids is the recently discovered bacterial endosymbiont *H. defensa*, which interferes with successful development of *Aphidius ervi* in the pea aphid *A. pisum* (Oliver et al. 2003). We found *H. defensa* in *A. craccivora*, as have previous studies (Russell et al. 2003; Oliver et al. 2005). Although our results do not prove that *H. defensa* conferred resistance to *B. communis* in *A. craccivora*, the pattern of developmental mortality in this species pair matched the pattern found in the *A. ervi*—pea aphid system. In both cases, parasitoid mortality occurs in the egg to early larval stages. In our study, *A. craccivora* was the only aphid with this pattern of mortality and also the only aphid to test positive for *H. defensa*.

Toxins within aphids can also interfere with successful development of immature parasitoids. In several hosts, high mortality occurred during the larval stage suggesting that parasitoids died from ingestion of unsuitable food. Aphids are known to sequester secondary metabolites when feeding on toxic plants (Mooney et al. 2008; Pratt et al. 2008) and these compounds can be detrimental to aphid natural enemies (Martos et al. 1992; Fuentes-Contreras et al. 1996; Helms et al. 2004). *B. communis* successfully completed development on *A. asclepiadis* when this aphid fed on morning glory whereas it suffered high mortality during the larval stage when the aphids fed on milkweed. These results suggest that milkweed toxins limited the suitability of *A. asclepiadis* as a host of *B. communis* during these experiments. An alternative explanation is that a selective sweep of the aphids occurred during the host switch to morning glory that also resulted in the aphids being a more benign host for *B. communis*.

Parasitoid larval mortality also increased in *A. nerii* on a medium-cardenolide milkweed plant compared to a low-cardenolide milkweed plant. Milkweed plants synthesize cardenolides which are sequestered by aphids (Mooney et al. 2008) and these cardenolides affect the survival and fitness of various natural enemies of aphids (Pasteels 1978; Malcolm 1989), although effects on aphid parasitoids have not been carefully examined. However, Helms et al. (2004) concluded that, based upon demographic studies, differences in cardenolide concentrations among milkweed plant species likely affect fitness of aphid parasitoids attacking *A. nerii*. The cardenolides in milkweed are a likely explanation for mortality of *B. communis* larvae in *A. asclepiadis* and *A. nerii*, as both aphids were reared on milkweed plants. *A. nerii* sequesters 25% more cardenolides than does *A. asclepiadis* and is attacked 50% less than *A. asclepiadis* is when both aphids are fed on the medium-cardenolide milkweed plant *A. syriaca* (Mooney et al. 2008). This is consistent with our finding of lower suitability of *A. nerii* than *A. asclepiadis* for *B. communis*. Low suitability on milkweed-feeding aphids may also result from other chemical defenses such as the steroidal pregnane glycosides (Warashina and Noro 2000). The relatively low larval survival of *B. communis* in *S. graminum* may result from secondary metabolites produced by some barley cultivars that may indirectly impact aphid parasitoids (Fuentes-Contreras et al. 1996).

In the absence of endosymbionts or secondary plant metabolites, mortality of parasitoids at the mummy stage in some aphid species (*A. monardae*, *A. oestlundi*, *A. rumicis* and *S. graminun*) may have resulted from low nutritional quality for parasitoid development. Parasitoid larvae have nutritional needs that are remarkably stage-specific as a result of the complex pathways of nutritional physiology associated with the parasitic lifestyle (Godfray 1994; Jervis et al. 2008). In aphidiine parasitoids, teratocytes injected during oviposition cause considerable redirection of metabolic physiology of the host and its associated mutualistic bacterial symbionts (bacteriocytes) to meet the demands of developing larvae (Falabella et al. 2000). However, the teratocyte-bacteriocyte interaction may be suboptimal in a novel host if physiology diverges too much from the original host (Pennacchio et al. 1999; Rahbé et al. 2002).

The sex ratios in experiment 1 differed from those obtained during experiment 3. In experiment 1, a broad range of host sizes was available and female-biased sex ratios were found only in the soybean aphid, *A. glycines*. The species with male-biased sex ratios are relatively small in size, and this may have led to a male-biased sex ratio in these species (Henry et al. 2006).

Aphid phylogeny and new associations

Many aspects of *B. communis* host use appeared to be related to the phylogenetic proximity of aphid species to *A. glycines*, the most preferred host in these experiments. Numbers of mummies (Fig. 1), propensity to sting hosts (Table 2), and suitability of hosts for development (Fig. 3) all appeared to be correlated with relatedness of aphids to *A. glycines*. This trend included some aphid species that are new associations for *B. communis*: both *A. monardae* and *A. oestlundi* are native to North America and proved to be somewhat suitable hosts in the laboratory environment. *S. graminum* stands out as a moderately suitable host despite not being in the genus *Aphis*, but this species is still in the same tribe as *Aphis*, the Aphidini. Within *Aphis*, exceptions to the phylogenetic trend involved aphids on plants with toxic plant compounds and an aphid with an endosymbiont implicated in reducing parasitoid fitness.

Conclusion

The experimental approach followed in our study examined host specificity and potential host range rather than the expected range of hosts attacked in the field. We determined that *B. communis* specialization may result from both physiological and behavioral constraints, but ecological factors such as spatial/temporal overlap and refuges from parasitism may act to narrow the actual host range in the field (Wyckhuys et al. 2007b, 2009). The parasitoid's host range involves primarily certain *Aphis* species that are not protected by endosymbionts or host-plant associations. Two-thirds of the aphid species that *B. communis* detects are accepted for oviposition, but the range of species that are ultimately successfully parasitized is further restricted by behavioral and physiological incompatibility of some host species as well as resistance incurred through association with toxic host plants and endosymbionts.

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